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Intestinal organoid cultures

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An abbreviated version of this protocol was published in Science in Mar 2021

γδ T cells regulate the intestinal response to nutrient sensing

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Detailed protocol

Z Sullivan Organoid Culture
Medzhtiov Lab, Feb 2018

Adapted from Sato et al *Nature 2009* and Sato et al *Gastroenterology* 2011

Basal organoid medium

500mL advanced DMEM/F12
100U/mL Penicillinl/Streptomycin (100X)
10mM HEPES (100X)
200mM GlutaMAX (100X)
N2 supplement (100X)
B27 supplement (50X)
1mM N-acetylcysteine (500X 81.6mg/mL stock in dH2O)

Store at 4 degrees for ~4 weeks

ENR medium

50mL basal medium
50ng/mL rmEGF (10,000X 500ug/mL stock in 0.1% BSA/PBS)
100ng/mL rmNoggin (1000X 100ug/mL stock in 0.1% BSA/PBS)
1ug/mL rhRspo1 (1000X 1mg/mL stock in 0.1% BSA/PBS)

Store at 4 degrees for 1 week

Crypt isolation

1. Isolate 20cm of small intestine and open longitudinally, rinse in cold PBS
2. Chop tissue into 2-5mm pieces and transfer to 25mL 2mM EDTA/PBS
    a. Incubate 30 minutes on ice
    b. During incubation, prepare ENR medium
    c. Prewarm 24-well non-TC treated plate
3. Remove EDTA medium with 10mL pipette
4. Resuspend tissues vigorously in 20mL cold 0.1%BSA/PBS using 10mL pipette for >30s
    a. Let settle and remove supernatant (villous fraction)
5. Reuspend pellet vigorously in 10mL cold 0.1%BSA/PBS using 10mL pipette and shake for 30s. Pass through 70uM strainer
6. Centrifuge at 200x g for 3 minutes at 4 degrees and gently discard supernatant
    a. Prewarm 24-well non-TC treated plate at 37 degrees
    b. Place Matrigel on ice
7. Resuspend crypts in 10mL cold basal medium, transfer to 15mL conical, and place on ice
    a. count crypts using hemocytometer
    b. aim to plate ~500 crypts per well
8. Centrifuge 200x g for 3 minutes at 4 degrees and remove supernatant
9. Resuspend crypts at a concentration of 500 crypts/100uL in 50% ENR medium/50% Matrigel
10. Quickly plate 50uL/well in pre-warmed 24-well non-TC treated plate
11. Incubate at 37 degrees for 10 minutes to set Matrigel
12. Add 500uL ENR medium and change medium 3x per week

Organoid maintenance

Passage organoids 1:5 once per week

1. Remove culture medium and add 500uL basal medium
    a. Thaw Matrigel on ice and pre-warm 24-well non-TC treated plate
2. Use 1000uL pipette to mechanically disrupt organoids + matrigel and transfer to 15mL falcon tube
    a. Further dissociate organoids using fire-polished Pasteur pipette
3. Add 10mL basal medium and centrifuge at 200x g for 2 minutes
4. Discard supernatant and resuspend in 125uL ENR medium + 125uL Matrigel
5. Quickly plate 50uL/well in pre-warmed 24-well non-TC treated plate
6. Incubate at 37 degrees for 10 minutes to set Matrigel
7. Add 500uL ENR medium and change medium 3x per week

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Sullivan, Z. and Medzhitov, R. (2021). Intestinal organoid cultures. Bio-protocol Preprint. bio-protocol.org/prep1098.

2. Sullivan, Z. A., Khoury-Hanold, W., Lim, J., Smillie, C., Biton, M., Reis, B. S., Zwick, R. K., Pope, S. D., Israni-Winger, K., Parsa, R., Philip, N. H., Rashed, S., Palm, N., Wang, A., Mucida, D., Regev, A. and Medzhitov, R.(2021). γδ T cells regulate the intestinal response to nutrient sensing. Science 371(6535). DOI: 10.1126/science.aba8310